IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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B. Webb
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Atty. Docket: GERSHONI=5

In re Application of:	)	Art Unit: 1655
Jonathan GERSHONI et al	)	Examiner: B. Forman
Appln. No.: 09/297,668	)	Washington, D.C.
Filed: May 6, 1999	)	September 12, 2001
For: DETERMINATION AND CONTROL OF BIMOLECULAR INTERACTIONS	)	

AMENDMENT

Honorable Commissioner for Patents
Washington, D.C. 20231

Sir:

In response to the Office Action of April 12, 2001, petition for a two-month extension of time and payment being attached to the RCE, please amend as follows:

IN THE SPECIFICATION

Delete the paragraph beginning at line 21, page 20, and extending through numbered line 9 on page 21 and replace with the following amended paragraph.

D1

One advantage of deriving pepscans from genetic material is that the sequence of the genetic material does not need to be known. As long as DNA is available, it can be digested and expressed in a phage display library, which can include up to from about 10^6 to about 10^{10} phages. The size

limitations of such a library easily accommodate even rather large genomes, including those of viruses, bacteria, yeast and parasites. For example, viruses have genomes of from about 10^3 to about 10^4 bp or more. Bacteria have genomes of about 10^6 bp, yeast about 10^7 bp and parasites from about 10^7 bp to about 10^8 bp or more. The size of the library required to accommodate a complete pepscan of the genome of an organism can be calculated according to the following formulas.

D' cont

$$number\ of\ peptides = \frac{genome\ size}{3}$$

$$chances\ of\ an\ inserted\ fragment\ yielding\ a\ viable\ phage = \frac{1}{3} * \frac{1}{3} * \frac{1}{2}$$

$$number\ of\ phages = \frac{genome\ size * 18}{3} = 6 * genome\ size$$

Of the number of phages initially required for insertion of DNA fragments, only 1/18 will be viable for an approximately single-fold coverage of the complete pepscan. Greater coverage, such as from about five- to about ten-fold coverage, is preferable. In any case, a complete pepscan of even the entire genome of a parasite of about 10^8 bp can clearly be accommodated by a single phage display library, since using the above formulas, only about $6*10^8$ phages would be required.

IN THE CLAIMS

Delete claims 112-143 without prejudice to the continuation of prosecution thereof in this or a divisional application.

144 (New). A method of identifying and producing a peptide which interacts with a ligand which interacts with a discontinuous epitope of a single biological unit, the method comprising:

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(a) providing a plurality of DNA fragments, which fragments appear in a DNA sequence which encodes said single biological unit;

(b) creating a library of oligonucleotides, each said oligonucleotide comprising at least two of said fragments, said fragments being randomly ligated;

(c) inserting each of said oligonucleotides into an expression system;

(d) causing the peptides encoded by said oligonucleotides to be expressed;

(e) screening the expressed peptides for interaction with a ligand that interacts with a discontinuous epitope of said single biological unit;

(f) identifying any peptide which so interacts; and

(g) producing any peptide so identified.

145 (New). A method in accordance with claim 144, wherein said procedure of (a) comprises cutting said DNA sequence to form said plurality of DNA fragments.

146 (New). A method in accordance with claim 145, wherein said cutting is accomplished enzymatically.

147 (New). A method in accordance with claim 145, wherein said cutting is accomplished mechanically.

D2 cont

148 (New). A method in accordance with claim 144, wherein said procedure of (a) comprises synthesizing said plurality of DNA fragments.

149 (New). A method in accordance with claim 144, wherein said procedure of (b) comprises randomly ligating said plurality of DNA fragments to form at least one ligated fragment and at least partially digesting said at least one ligated fragment to form said library of oligonucleotides.

150 (New). A method in accordance with claim 144, wherein said expression system comprises a plurality of bacteria and said procedure of (c) comprises inserting one of said library of oligonucleotides into each of said plurality of bacteria.

151 (New). A method in accordance with claim 144, wherein said expression system comprises a plurality of phages and said procedure of (c) comprises inserting one of said library of oligonucleotides into each of said plurality of phages.

152 (New). A method in accordance with claim 151, wherein said oligonucleotides are inserted into said phages by cloning said oligonucleotides into phage genes coding for a coat protein.

153 (New). A method in accordance with claim 152, wherein said phages are filamentous phages and said coat protein is pIII or pVIII.

D2 cont

154 (New). A method in accordance with claim 144, wherein said expression system comprises an eukaryotic expression system and said procedure of (c) comprises inserting said library of oligonucleotides into eukaryotic expression vectors and inserting said vectors into said eukaryotic expression system.

155 (New). A method in accordance with claim 144, wherein said single biological unit is a protein.

156 (New). A method in accordance with claim 144, wherein said single biological unit is two or more proteins which interact to form a complex.

157 (New). A method of vaccinating a subject against an organism, comprising placing a product produced in accordance with the method of claim 144 into a vaccine carrier and administering said product and vaccine carrier to the subject.

158 (New). A library of peptides, each of which comprises at least two peptide fragments, each said fragment

appearing in the amino acid sequence of a single biological unit, said fragments being randomly ligated to form said peptides.

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159 (New). A method of preparing a library of peptides which can be screened to find peptides which interact with ligands which interact with discontinuous epitopes of a single biological unit, comprising:

(a) providing a plurality of DNA fragments, which fragments appear in a DNA sequence which encodes said single biological unit;

(b) creating a library of oligonucleotides, each said oligonucleotide comprising at least two of said fragments, said fragments being randomly ligated;

(c) inserting each of said oligonucleotides into an expression system; and

(d) causing the peptides encoded by said oligonucleotides to be expressed.

160 (New). A method in accordance with claim 159, wherein said procedure of (a) comprises cutting said DNA sequence to form said plurality of DNA fragments.

161 (New). A method in accordance with claim 160, wherein said cutting is accomplished enzymatically.

162 (New). A method in accordance with claim 160, wherein said cutting is accomplished mechanically.

163 (New). A method in accordance with claim 159, wherein said procedure of (b) comprises randomly ligating said plurality of DNA fragments to form at least one ligated fragment and at least partially digesting said at least one ligated fragment to form said library of oligonucleotides.

164 (New). A method in accordance with claim 159, wherein said expression system comprises a plurality of bacteria and said procedure of (c) comprises inserting one of said library of oligonucleotides into each of said plurality of bacteria.

D2 cont

165 (New). A method in accordance with claim 159, wherein said expression system comprises a plurality of phages and said procedure of (c) comprises inserting one of said library of oligonucleotides into each of said plurality of phages.

166 (New). A method in accordance with claim 165, wherein said oligonucleotides are inserted into said phages by cloning said oligonucleotides into phage genes coding for a coat protein.

167 (New). A method in accordance with claim 166, wherein said phages are filamentous phages and said coat protein is pIII or pVIII.

168 (New). A method in accordance with claim 159, wherein said expression system comprises an eukaryotic expression system and said procedure of (c) comprises

inserting said library of oligonucleotides into eukaryotic expression vectors and inserting said vectors into said eukaryotic expression system.

169 (New). A method in accordance with claim 159, wherein said single biological unit is a protein.

D2 cont

170 (New). A method in accordance with claim 159, wherein said single biological unit is two or more proteins which interact to form a complex.

171 (New). A method of identifying and producing an oligonucleotide which interacts with a ligand which interacts with a discontinuous epitope of a single biological DNA or RNA unit, the method comprising:

(a) providing a plurality of DNA fragments, which fragments appear in a DNA sequence of a single biological DNA unit or correspond to an RNA sequence of a single biological RNA unit;

(b) creating a library of oligonucleotides, each said oligonucleotide comprising at least two of said fragments, said fragments being randomly ligated;

(c) if the biological unit is an RNA unit, transcribing the DNA of each of said oligonucleotides to RNA to form an RNA oligonucleotide library;

(d) screening the oligonucleotide library for interaction with a ligand that interacts with a discontinuous epitope of said single biological unit;

(e) identifying any oligonucleotide which so interacts; and

D2 cont

(f) producing any oligonucleotide so identified.

172 (New). A method in accordance with claim 171, wherein said single biological unit is a telomere.

173 (New). A method in accordance with claim 171, wherein said single biological unit is a tRNA.

174 (New). A method in accordance with claim 171, wherein said single biological unit is a ribozyme.

175 (New). A method of preparing a library of oligonucleotides which can be screened to find oligonucleotides which interact with ligands which interact with discontinuous epitopes of a single biological DNA or RNA unit, comprising:

(a) providing a plurality of DNA fragments, which fragments appear in a DNA sequence of a single biological DNA unit or correspond to an RNA sequence of a single biological RNA unit;

(b) creating a library of oligonucleotides, each said oligonucleotide comprising at least two of

(c) if the biological unit is an RNA unit, transcribing the DNA of each of said oligonucleotides to RNA to form an RNA oligonucleotide library.

D2 Concl'd

176 (New). A library of oligonucleotides, each of which comprises at least two DNA fragments, wherein each of said fragments appear in a DNA sequence which comprises, encodes or transcribes a single biological unit, said fragments being randomly ligated to form said oligonucleotides.

REMARKS

Claims 144-176 presently appear in this case. No claims have been allowed. The official action of April 12, 2001, has now been carefully studied. Reconsideration and allowance are hereby respectfully urged.

Briefly, the present invention relates to a method for identifying continuous peptides which simulate a discontinuous epitope of a single biological unit, i.e., which interact with a ligand which interacts with a discontinuous epitope of a single biological unit. The single biological unit may be a protein or a complex of proteins. It may also be a DNA or RNA unit. The DNA, which may be the DNA that comprises the biological unit or that corresponds to the RNA of the biological unit or that encodes the amino acid sequence of a proteinaceous biological unit, is divided into DNA fragments. A library of oligonucleotides, each comprising at least two of such fragments that are randomly ligated, is then created. Preferably, this library will contain oligonucleotides of fragment pairs in which each fragment is linked to each other fragment. If the biological unit is a protein or a complex of proteins, the oligonucleotides are inserted into an expression system and then expressed. If the biological unit is an RNA unit, the DNA is then transcribed to the corresponding RNA. The resultant is then screened for

interaction with a ligand that interacts with a discontinuous epitope of the single biological unit. Those that are identified with such positive interaction are then produced and can serve to simulate the native discontinuous epitope.

The interview among Examiners Forman and Zitomer, the inventor, Prof. Jonathan Gershoni, and the undersigned attorney for applicants, conducted on September 7, 2001, is hereby gratefully acknowledged. In the course of this interview, the references applied in the rejections of record were discussed and applicants explained the differences between the random ligation of fragments in the present invention and the non-random ligation of fragments in Gritz. It was further explained that Mandeville involved used of oligonucleotides whose sequence is totally random, while the present invention involved the random ligation of fragments of the biological unit of interest. New claim language was discussed to more clearly define the invention, with additional steps of ligating random fragments and selection of epitopes. The present amendment presents a new set of claims based on the language discussed at the interview. The arguments made at the interview will be substantially repeated herein.

Claims 137-143 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Gritz in view of Mandeville. The examiner states that Gritz teaches a method for preparing a peptide of a discontinuous epitope of a single

biological unit of an organism, such as the HIV env gene, the method including the steps of providing a plurality of DNA fragments corresponding to at least a portion of a genome by digesting the genome to form the plurality of fragments, ligating to form at least one ligated fragment, at least partially digesting the at least one ligated fragment to form a plurality of fragments coding for the discontinuous epitope, inserting the discontinuous epitope into an expression system, and obtaining a peptide from the expression system. The examiner states that a number of different recombination events occur to form a discontinuous library, i.e., a diverse set of chimeric env genes. The examiner recognizes that Gritz does not teach forming the discontinuous library prior to inserting the library into the expression system. The examiner states that Mandeville teaches a similar method for preparing a conformational peptide of a discontinuous epitope which includes providing a plurality of DNA fragments corresponding to at least a portion of a genome of an organism, ligating the fragments, inserting the library into the an expression system, and obtaining the peptide from the expression system. The examiner considers it to have been *prima facie* obvious to modify the insertion, followed by recombination, to form the discontinuous library of Gritz with the direct insertion of the discontinuous epitopes 5' to a coat protein, such that expressed epitopes are displayed as a

portion of an outer structural protein of the bacteriophage for the expected benefit of screening the displayed epitopes to identify the best diversity of epitopes binding to any ligand and using the displayed discontinuous epitopes as an immunogen to produce antibodies as taught by Mandeville. The examiner's comments about the dependent claims will not be discussed here as the allowability of independent claims should render the dependent claims also patentable for the same reason. This rejection is respectfully traversed.

There are very basic differences between the procedure of Gritz and that of the present invention. Gritz starts with two or more DNA sequences that share regions of homology (column 5, lines 52-53). Gritz makes clear that a certain degree of sequence homology is necessary in order to direct recombination between related DNA sequences. Gritz digests the two homologous DNA sequences. All of these sequences are inserted into the DNA genome of the host by homologous recombination. Thus, the resultant product is the entire gene in the required order but being chimeric in that it has portions from each of the two starting genes. The intent is that the final product be functionally relevant. Thus, Gritz does not display randomly ligated combinations of fragments as with the present invention. It is a basically different intent and process.

In order to more particularly point out and distinctly claim that which applicants consider to be their invention, the present amendment presents a new set of claims. In claim 144, procedure (b) requires:

> creating a library of oligonucleotides, each said oligonucleotide comprising at least two of said fragments, said fragments being randomly ligated.

Gritz does not create a library of oligonucleotides from randomly ligated fragments. In the present invention this is done so as to obtain a library in which each fragment from the DNA sequence which encodes the "single biological unit" is ligated to each other fragment so that every possible combination of fragments will occur. It is expected that one or more of these fragments will simulate a discontinuous epitope of the original biological unit. In other words, when the biological unit is folded so that two different portions of its sequence are in proximity in the folded structure, those two portions of the original sequence, when ligated together, will be recognized by the same ligand that recognizes the discontinuous epitope formed when these fragments are in proximity but not physically ligated. This is very different from the procedure of Gritz, and it would not be obvious to anyone of ordinary skill in the art reading Gritz to perform such a random ligation.

Furthermore, procedure (e) of claim 144 requires:

screening the expressed peptides for interaction with a ligand that interacts with a discontinuous epitope of said single biological unit.

Gritz performs no such screening. Gritz screens for functional proteins. This is another step that distinguishes the procedure of the present invention from the distinctly different procedure of Gritz.

Mandeville is more similar to the procedure of the present invention in that it also attempts to find peptides that simulate discontinuous epitopes of proteins. However, Mandeville does this in a distinctly different way from that of the present invention. Mandeville does not provide a plurality of DNA fragments which appear in a DNA sequence which encodes a single biological unit (procedure (a) of claim 144). Mandeville makes completely random oligonucleotide sequences in order to randomly locate peptides that simulate discontinuous epitopes. However, it would require phages in orders of magnitude larger than that physically possible in order to create every random peptide having a length of ten amino acids, which is indicated to be preferred by Mandeville. The present invention is based on an improved process which does not require totally random generation of peptides. By the present invention, one starts with the sequence of the protein of interest. One then divides the DNA sequence encoding that protein into fragments and randomly ligates the

fragments into lengths of at least two fragments. In this way, a standard phage expression library can be expected to contain every possible pair of fragments, i.e., each fragment paired with each other fragment. This is a more rational design of peptides intended to simulate discontinuous epitopes, which rational design is not suggested or made obvious by Mandeville or any combination of Mandeville with Gritz.

Accordingly, neither Gritz nor Mandeville nor any combination thereof suggests the procedure (b) of claim 144 presented herewith. The other differences discussed above are also significant, but it is the random ligation of fragments that is the crux of the present invention. As this is neither taught nor suggested by either reference alone or by any combination thereof, reconsideration and withdrawal of this rejection is respectfully urged.

It should be noted that the term "biological unit" is defined, for example, at page 33, lines 18-21. The screening step is disclosed, for example, in the paragraph bridging pages 49 and 50. See also the screens set forth in Example 8. Accordingly, all the features of all of the present claims are fully supported by the present specification. Entry, consideration and allowance of each of these claims is, therefore, respectfully urged.

Pages 20 and 21 of the present specification have been amended in order to correct clerical or mechanical errors. At page 20, line 24, obviously "from about 10^{10} to 10^{10} phages" was not intended. The first number has been changed to "10^{6}" as this is the order of magnitude that is supported, for example, at page 22, line 17. As to the correction to page 21, it is clear from context that lines 4 and 3 were inverted during the word processing procedure. Accordingly, no new matter is involved in correcting this obvious error.

It is submitted that all the claims now present in the case clearly define over the references of record and fully comply with 35 U.S.C. §112. Reconsideration and allowance are, therefore, earnestly solicited.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made".

Respectfully submitted,

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